other mouse models of CRC are analyzed at adult stages (>8 weeks). It would be important to confirm these findings using different CRC models (e.g., *Smad3*$^{-/-}$) and also to determine whether the deleterious effect of butyrate is mediated through G protein-coupled receptors (Jobin, 2014). For example, reducing butyrate levels through dietary manipulation (fiber) or molecular deletion (*Gpr109a*$^{-/-}$) augmented polyp formation in *Apc*$^{Min/+}$ mice, a phenomenon linked to decreased T regulatory (Treg) cell differentiation (Singh et al., 2014).

It is worth noting that this is not the first instance of a "butyrate paradox." Butyrate has long been known to have differential effects on normal versus cancerous colonocytes, and only recently has this been addressed. Due to the Warburg effect, butyrate is metabolized by cancerous colonocytes to a lesser extent and therefore accumulates as an HDAC inhibitor (Donohoe et al., 2012). Similarly, butyrate may have heterogeneous effects on tumorigenesis depending on host genetic background, the presence of other bacterial metabolites such as an omega-3 fatty acid (docosahexaenoic acid), which synergizes with butyrate to induce colonocyte apoptosis (Kolar et al., 2007), and whether it is exerting a direct effect on the tumor (cell autonomous) versus non-cell-autonomous effects such as regulating mucosal immune cell activity as mentioned above. Therefore, although the current study contributes to our understanding of the interplay between diet, microbes, and CRC, the role of butyrate in cancer protection/promotion will still require further investigation. Altering microbial activities through dietary manipulation represents an exciting means to harness the microbiome and influence health and disease states. Whether dietary manipulation could be used effectively to preserve homeostatic functions afforded by microbiota while attenuating its potential pathological effects is still an open question, and more research would be necessary before this strategy becomes a reality.

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What Lies Within: Coinfections and Immunity

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Helminth-induced immunomodulation is thought to influence the outcome of secondary infections. Osborne et al. (2014) and Reese et al. (2014) demonstrate that helminth infection impacts viral infections by tilting the immune system toward Th2/M2 immune regulatory responses that dampen Th1/M1 antiviral responses as well as promote reactivation of latent herpesviruses.

The mammalian intestine is home to many pathogens, including commensal bacteria, helminth parasites, and viruses. Among these, helminths represent some of the earliest recorded human infections in history and remain a significant source of infection today. Approximately 2.7 billion people who live in low-income countries in Africa, South America, and Asia are thought to have some type of helminth infection (Hotez et al., 2007). In addition to infectious complications, helminths are also associated with human malignancy. *S. hematobium* is a platyhelminth that is associated with bladder cancer, particularly in Egypt. Additionally, *O. viverrini* (liver fluke) and *C. sinesis* are classified as group 1 carcinogens by the International Agency for Research on Cancer (IARC) (Bouvard et al., 2009) and are causally associated with cholangiocarcinoma, which is highly prevalent throughout much of southeast Asia and Egypt. The presumed mechanisms include chronic inflammation and hyperplasia of billary epithelium.

Helminth-induced immunomodulation has long been thought to influence human

immunity either in a direct fashion or indirectly through modulation of the intestinal microbiota. The type 2 response is the arm of the immune system that minimizes the pathogenesis associated with helminth infection but also plays key roles in immune disorders such as asthma and allergies. Type 2 responses are characterized by the induction of CD4+ T helper (Th) 2 cells and alternatively activated macrophages (AAMacs) or type 2 macrophages (M2). The Th2 response is triggered by cytokines; foremost among them are interleukin-4 (IL-4) and IL-13 in addition to IL-5 and IL-9. Through the production of IL-4, Th2 cells help B cells secrete immunoglobulin E (IgE). B cells respond to IL-4 via activation of the IL-4Rα/IL-4Rγ receptor (type I). Macrophages express multiple IL-4R subunits (type I and type II), which allow them to respond to IL-4 as well as IL-13, which can also bind the IL-4R. Upon receptor activation, STAT6 becomes phosphorylated and moves into the nucleus and binds to the promoter elements of STAT6 responsive genes. In contrast, Th1 cell responses, which are generated in response to most viral infections, result in the secretion of gamma interferon (IFN-γ), activation of macrophages to type 1 macrophages (M1), and the activation of CD8+ cytotoxic T cells that can kill virus-infected cells. These Th1 and Th2 responses can act in opposition. In particular, IFN-γ and IL-4 act antagonistically.

Since helminth infection has such a profound polarizing effect on the mammalian immune system locally and beyond the gut-associated lymphoid environment, it provides a clinically relevant model to ask questions about polymicrobial interactions: if and how infection with one pathogen predisposes the host to respond to subsequent infection with a second, unrelated pathogen and whether the life cycle of the secondary pathogen is altered in any way. The papers by Osborne et al. (2014) and Reese et al. (2014) have examined these questions in the context of helminth-virus coinfections.

To determine whether helminth infection directly impacts host immunity or whether it indirectly affects immunity via the gut microbiota, Osborne and colleagues developed a model of enteric coinfection using *Trichinella spiralis* (Ts), a nematode, which inhabits the small intestine, and a murine norovirus (MNV) that infects the ileum. They report that infection with Ts elicited a long-lasting inhibition of antiviral immunity against MNV as well as influenza, a virus that homes to the respiratory tract. Thus, helminth-elicited immunomodulation of antiviral immunity exists beyond the local environment of the intestine and can impact antiviral immunity against virus-infected cells at distal sites in the human host.

Several theories suggest that, in addition to direct effects on macrophages and CD4+ T cells, helminth infection also modulates host immunity through effects on the intestinal microbiota. Osborne et al. (2014) performed sequencing and phylogenetic analysis of bacterial 16S rRNA genes of the GI tract following Ts infection and found that alterations in the intestinal microbiota did indeed occur post-Ts infection. However, the degree of impairment of antiviral responses against MNV in helminth-infected conventional and germ-free mice was very similar, revealing that helminth infection impacts host immune responses directly and was not dependent on the microbiota.

The Th2 cytokines induced by helminth infection are linked to STAT6-dependent AAMacs, or M2, resulting in the induction of several AAMac-marker molecules, including arginase 1 (Arg1) and Ym1 (Chi3l3). Arg1 converts arginine to ornithine and urea. Since arginine is also needed to produce nitric oxide (NO), one outcome of inducing Arg1 is a dampened macrophage response to intracellular pathogens. Ym1 is a chitinase-like protein that is highly upregulated during Th2 responses. Ym1 is a secreted protein and hence can modulate the immune effects of intestinal infection in a systemic fashion. Osborne et al. discovered that the AAMacs pathway as well as Arg1 and Ym1 gene expression were activated in the intestines of wild-type Ts- and MNV-coinfected mice but not STAT6- or IL-4Rα-deficient animals (Figure 1). The AAMacs pathway induced upon helminth infection dampens antiviral responses in a STAT6- and Th2 cytokine-dependent manner. The Ts-induced M2 response was dominant over the MNV-induced M1 response, although this may be because helminth infection occurred prior to MNV infection. Furthermore, these responses were dependent on IL-4, a cytokine known to activate the AAMacs pathway, even at early times post-MNV infection.

Antibody-mediated neutralization of Ym1 in Ts- and MNV-coinfected mice resulted in increased CD8+ T cell responses and increased numbers of virus-specific CD8+ T cells resulting in enhanced control of viral replication. Taken together, it appears that helminth infection directly modulates host immunity to hinder responses against viral infection, even at sites of viral infection distant from the helminth-infected intestine. Such immunomodulation is independent of the gut microbiota but is dependent on the activation of the AAMacs pathway and the inhibition of CD8+ antiviral T cell responses through the action of the Ym1 molecule.

In a related study, Reese et al. (2014) show that helminth-induced Th2 polarization can promote viral infections via a different mechanism. Using helminth-infected mice that were latently infected with murine gammaherpesvirus 68 (MHV-68), the authors show that the canonical Th2 and M2 response to helminth infection (Van Dyken and Locksley, 2013) can reactivate latent MHV-68 (Figure 1). This model system allows for exceptional genetic tools to analyze the tripartite virus-parasite-host interactions that cannot be easily studied with the human-tropic gammaherpesviruses, Kaposi sarcoma-associated herpesvirus (KSHV), and Epstein-Barr virus (EBV).

Reese et al. use different MHV-68 mutant viruses to dissect the mechanism of multiple pathogen interactions in the host. Helminth infection induces the Th2 cytokine IL-4, which engages the viral transactivator Rta/Orf50 via Stat6. IL-13, which also binds the IL-4Rα chain, and Stat6 had the same phenotypic effect on MHV-68-infected macrophages. In contrast, helminth infection of latently infected Stat6 knockout mice did not result in reactivation. Rta/Orf50 is necessary and sufficient to initiate virus reactivation, but MHV-68 reactivation is subject to negative regulation by IFN-γ. Both IL-4-mediated induction and liberation from IFN-γ suppression are needed in vivo for virus reactivation. Interestingly, the signaling network needed to induce MHV-68 reactivation represents only a subset of the signaling cascade needed for complete Th2 polarization of bone marrow macrophages, which supports the idea of a direct IL-4/Stat6/RTA promoter loop, rather than the virus responding to cellular reprogramming as in MHV-68 latently infected B cells (Siegel et al., 2010).

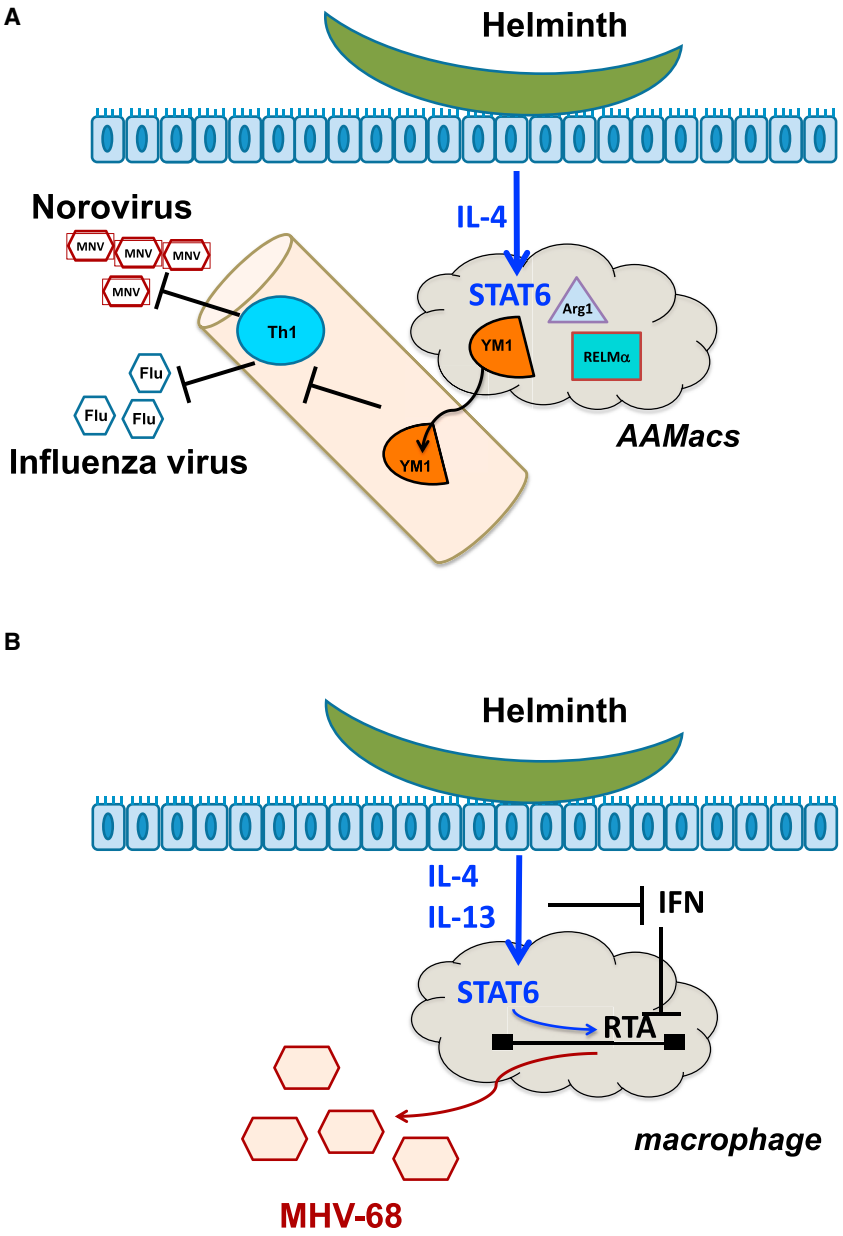

Figure 1. Helminth Infection Induces Type 2 Immune Responses and the Production of IL-4, which Effectively Induces the AAMacs Pathway

(A) Summary of the systemic release of YM1 in proximal macrophages and their reprogramming into AAMacs. This has local as well as systemic consequences resulting in increased susceptibility to intestinal (norovirus) and respiratory (influenza virus) infection.

(B) Reactivation of latent MHV-68 through a direct interaction between STAT6 and the promoter of the viral immediate early transactivator RTA (Orf50). IL-4/IL-13 counteracts IFN-γ, which normally restricts MHV-68 reactivation from macrophages.

IL-4 had a similar proreactivation phenotype in KSHV latently infected B lymphoma cells in culture, as does the Th1 cytokine, IFN-γ (Chang et al., 2000). This discordance with regard to IFN-γ is not surprising. B cells are not macrophages. They are hardwired to respond differently and to different stimuli. KSHV reactivation from latent B cells has been linked to IFN-γ activating the viral IL-6 promoter (Chatterjee et al., 2002) and TLR7/TLR8 activation by agonists as well as by secondary infection with vesicular stomatitis virus (Gregory et al., 2009; West et al., 2012).

Collectively, these two articles support the general idea of the vironome as an integral part of the host. The interrelationships between multiple pathogens within a host are very complex. A range of pathogenic outcomes have been observed with certain other coinfections such as HIV and tuberculosis (TB). Perhaps the best-known example with respect to the herpesviruses is that of EBV and malaria coinfection in Sub-Saharan Africa (Torgbor et al., 2014). The findings from Reese et al. and Osborne et al. suggest that helminth coinfections with viruses may also represent a significant source of mortality and morbidity in the human population.

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